Phytochemistry, 1977, Vol. 16, pp. 1359–1364. Pergamon Press. Printed in England.

DINITROPHENOL-INDUCED EFFLUX OF SUCROSE FROM MAIZE SCUTELLUM CELLS*

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(*Revised received* 7 *March* 1977)

Key Word Index—*Zea mays*; Gramineae; maize scutellum; sucrose efflux; dinitrophenol.

Abstract—Maize scutellum slices accumulated sucrose during incubation in glucose, fructose or sucrose. Sucrose was accumulated in two compartments, tentatively identified as vacuole and cytoplasm. 2,4-Dinitrophenol (DNP) caused an efflux of sucrose from both compartments and caused an influx of H^+ across the plasmalemma. Both fluxes increased as the pH was lowered over the range from 5 to 3.5, but pH had a much greater effect on H^+ influx than it did on sucrose efflux. At pH 5, there was no net H^+ influx although sucrose efflux continued. It is concluded that sucrose efflux across the plasmalemma does not involve a sucrose-H^+ cotransport system. Mannose treatment, which drastically reduces the ATP level of scutellum slices, did not induce either a H^+ influx or a sucrose efflux, although these fluxes occurred when mannose-treated slices were placed in DNP. It is concluded that the H^+ and sucrose fluxes were not the result of a low level of ATP.

INTRODUCTION

In bacterial systems, uncouplers of phosphorylation induce an efflux of accumulated metabolites, e.g. β-galactosides from *Escherichia coli* [1, 2]. Accumulation of β-galactosides is thought to result either from a direct interaction between the membrane carrier and sites of energy coupling [1, 3] or, according to the chemiosmotic hypothesis, from a metabolite-H^+ symport system driven by a pH difference across the membrane [2]. In either case, the system appears to degenerate to one of facilitated diffusion in the presence of an uncoupler.

In plant cells a different situation appears to exist; uncouplers such as 2,4-dinitrophenol (DNP) or carbonyl-cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) usually do not cause efflux of accumulated metabolites although they strongly inhibit the accumulation process itself [4–7]. Even in *Chlorella vulgaris*, where hexose transport can be described as a proton symport [8, 9], FCCP does not cause efflux of accumulated 3-*O*-methyl-glucose [5]. In contrast, Finkelman and Reinhold [10] obtained a DNP-induced leakage of reducing sugars from sunflower hypocotyl segments. In these cells the plasmalemma appears to offer little resistance to free diffusion of hexoses [11]. Therefore, DNP must have had its effect at the tonoplast.

In the present paper, it is shown that treatment of maize scutellum tissue with DNP causes an efflux of sucrose and an influx of H^+. Evidence is presented that sucrose efflux represents transport across both tonoplast and plasmalemma.

RESULTS

Sucrose accumulation

Scutellum slices accumulated sucrose when incubated in solutions of glucose, fructose or sucrose, and similar rates of sucrose accumulation were obtained with the 3 sugars (Fig. 1). After 5 hr, the sucrose concentration of the tissue was about 0.2 M, assuming 80% of the tissue fr. wt is intracellular water within which the sucrose is uniformly distributed. In contrast, the glucose content

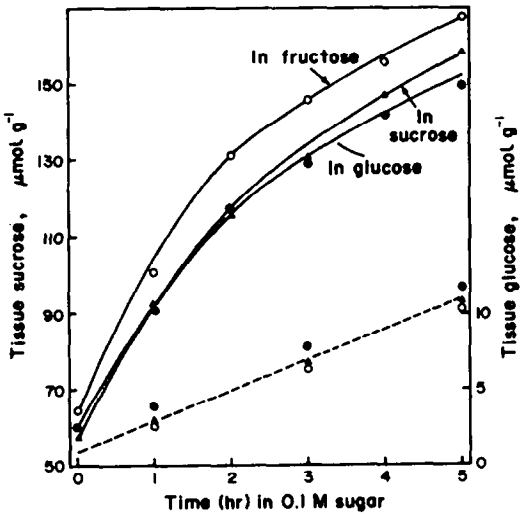

Fig. 1. Accumulation of sucrose and glucose during incubation of scutellum slices in fructose, glucose and sucrose. Groups of slices (0.5 g) were incubated for 30 min in H_2O and 30 min in a soln of KCl, $MgCl_2$, and $CaCl_2$ (each at a concn of 10 mM). Treatment in the salt soln minimized sucrose leakage during the subsequent sugar incubations [12]. The slices were then washed in H_2O and placed in 0.1 M sugar solns (zero time on the graph). The bathing solns were replaced with fr. sugar soln each hr. Groups of slices were killed and extracted in boiling 80% EtOH at the end of each hr. Slices incubated in glucose and sucrose were washed and incubated in H_2O for 20 min before killing in order to remove free space sugars. The graphs represent average values from at least two experiments with each sugar. Tissue sucrose at zero time varied with each crop of seedlings. Tissue glucose is shown by the dashed line.

* Florida Agricultural Experiment Station Journal Series No. 6149.

Table 1. Leakage of sucrose into water following incubation of slices in fructose and sorbitol*

Treatment	Sucrose, μmol/g	
	Tissue	Leakable
(1) 3 hr Fructose (M)	165	64
(2) 3 hr Fructose (M), 1 hr sorbitol (M)	165	65
(3) 3 hr Fructose (0.1 M)	152	3
(4) 0 hr Fructose, 1 hr sorbitol (M)	68	11
(5) 3 hr Fructose (0.1 M), 1 hr sorbitol (M)	154	15
(6) 7 hr Fructose (0.1 M), 1 hr sorbitol (M)	186	17

* Groups of slices (0.5 g) were incubated in H_2O followed by a salt soln (Fig. 1), and then they were washed and placed in the treatment solns. Fructose solns were replaced with fr. solns each hr. Before incubating in sorbitol, slices were rinsed with 10 ml of sorbitol. Two groups of slices were used for each treatment. At the end of the treatment period, slices were quickly rinsed with 10 ml of H_2O. Then, one group was killed in boiling 80% EtOH to obtain tissue sucrose and the other group was incubated in 10 ml of H_2O for 60 min to obtain leakable sucrose.

of the tissue, 1 to 2 μmol at zero time, increased to only 10 to 12 μmol after 5 hr in 0.1 M glucose, fructose or sucrose (Fig. 1). After each period of incubation in glucose or sucrose, the slices were rinsed with water and incubated in water for 20 min before alcohol extraction. Therefore, the tissue glucose was not in the free space [13].

It was shown previously [14–16] that some of the accumulated sucrose leaks from the slices when they are transferred from hexose solutions to water. The amount of leakage is negligible following incubation in 0.1 M hexose, but it increases as the hexose concentration is increased and becomes a significant fraction of the total sucrose content of the tissue. This is illustrated in Table 1. Slices transferred to water after 3 hr in 1.0 M or 0.1 M fructose leaked 64 μmol or 3 μmol respectively (Table 1, treatments 1 and 3). Since both groups of slices contained high levels of sucrose, it appeared that transfer to water from a concentrated solution was necessary for sucrose release. To test this, slices were incubated in 1.0 M sorbitol for 1 hr following the fructose incubation and before transfer to water. Sorbitol had no effect on sucrose leakage from slices that had been incubated in 1.0 M fructose (Table 1, treatments 1 and 2), but it increased the amount of sucrose (from 3 to 15 μmol) that leaked from slices that had been incubated in 0.1 M fructose (treatments 3 and 5). The 'M sorbitol' technique was used to measure the amount of sucrose in the leakable pool with time of incubation in 0.1 M fructose (Table 1, treatments 4, 5 and 6). Slices placed directly into sorbitol with no fructose incubation released 11 μmol of sucrose when transferred to water. This amount of sucrose is taken to be the size of the leakable pool in the fresh slices. When a 3 hr or 7 hr incubation in 0.1 M fructose preceded the sorbitol incubation, the amounts of sucrose that were released into water were 15 μmol or 17 μmol respectively. Thus, during 7 hr in 0.1 M fructose, the leakable sucrose pool increased by only 7 μmol whereas tissue sucrose increased by 118 μmol.

The results of Table 1 indicate that there are two pools of sucrose in the scutellum: a leakable pool and a non-leakable or stored pool. During incubation in 0.1 M fructose almost all of the newly synthesized sucrose entered the stored pool. During incubation in M fructose

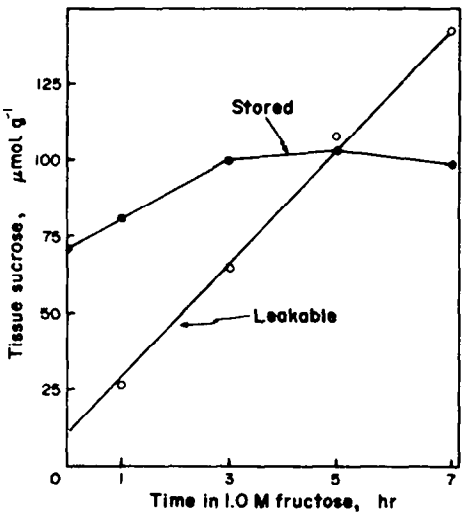

Fig. 2. The amounts of leakable and stored sucrose in scutellum slices with time of incubation in M fructose. Groups of slices (0.5 g) were given initial H_2O and salt soln incubations (Fig. 1), washed and placed in 10 ml of M fructose. The bathing solns were replaced with fr. fructose solns each hr. At the end of 1, 3, 5 and 7 hr, one group of slices was killed to determine total tissue sucrose, and one group was placed in H_2O in order to determine leakable sucrose (cf. Table 1); stored sucrose was calculated by difference. Leakable sucrose at zero time was determined by placing slices directly in M sorbitol (with no fructose incubation) for 1 hr and determining sucrose leakage after transferring them to H_2O (Table 1, treatment 4).

much of the newly synthesized sucrose entered the leakable pool. The amounts of sucrose in the two pools with time of incubation in M fructose are shown in Fig. 2. The amount of sucrose in the leakable pool increased linearly during the 7 hr of incubation. The amount of leakable sucrose at zero time was taken from the results of Table 1, treatment 4. The amount of stored sucrose increased during the first 3 hr and thereafter remained essentially constant. Results similar to those of Fig. 2 were obtained with M glucose. In contrast, slices placed in M sucrose became flaccid, they lost sucrose instead of gaining it; and, after being removed from the sucrose solution and rinsed in water, they were unable to synthesize sucrose when incubated in 0.1 M fructose.

The observation that hypertonic sucrose, but not hexose or hexitol, injured the scutellum cells can be explained by the fact that the scutellum plasmalemma is a negligible barrier to diffusion of hexoses and hexitols [13, 17] whereas sucrose appears to penetrate only by a specific transport mechanism [18–20]. The release of sucrose from the leakable pool (Table 1, Fig. 2) did not occur through the plasmalemma; rather, evidence has been presented [15, 16] that it leaves the slices through the cut ends of the phloem in a flow of solution driven by hydrostatic pressure.

In the next section, it is shown that DNP causes an efflux of sucrose from both of the sucrose compartments of the scutellum.

Efflux of sucrose in the presence of DNP

In this paper, the term 'leakage' means release of sucrose from the slices and it carries no connotation as to route, whereas the term 'efflux' (or influx) means movement across a membrane.

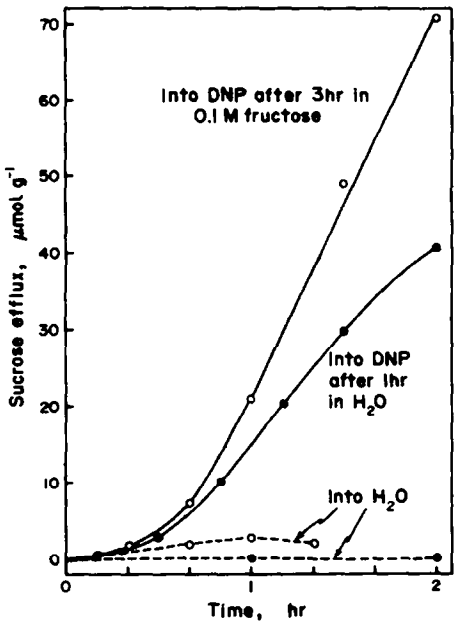

Fig. 3. Leakage of sucrose from scutellum slices incubated in DNP. Groups of slices (1 g) were incubated in H_2O for 1 hr or in 0.1 M fructose for 3 hr. The slices were then washed and placed in 17 ml of 0.5 mM DNP. Slices placed in 17 ml of H_2O served as controls. The DNP and H_2O solns were maintained at pH 4 with HCl. At zero time, slices pretreated in H_2O contained 73 μmol sucrose per g, and those pretreated in fructose contained 155 μmol per g.

There was a leakage of sucrose when slices were placed in a DNP solution maintained at pH 4. HCl was used to maintain pH because DNP induces a H^+ influx thereby causing the bathing solution pH to rise [21]. Little or no sucrose leakage occurred when control slices were placed in water at pH 4 (Fig. 3). Slices containing relatively high amounts of sucrose (prior incubation in 0.1 M fructose) and relatively low amounts of sucrose (prior incubation in water) were used in this experiment, and the rates of sucrose leakage reflect this difference in sucrose content (Fig. 3). Nevertheless, with both kinds of slice there was a lag period of 40 to 50 min before a constant rate of sucrose leakage was attained. Since the slices contained only about 11 to 15 μmol of sucrose in the leakable pool (cf. Table 1), the lag period might represent the time required for the release of sucrose from the stored pool into the leakable pool.

Table 2. Sucrose leakage from scutella of intact seedlings*

Treatment	Sucrose leaked, μmol/12 scutella
Seedlings intact; scutella in H_2O	7.6
Seedlings intact; scutella in DNP	31.5
Scutella excised into H_2O	46.0

* Each of 36 seedlings (minus ensodperm) was placed in a plastic tube so that the scutellum (abaxial surface down) rested on the bottom, and the primary root and the shoot were bent upward along the wall of the tube. Enough fructose soln (M) was added to reach but not cover the scutellar node. The tubes were placed in a moist atmosphere in the dark at about 24°. After 3 hr, the fructose was removed and H_2O (12 seedlings) or 0.5 mM DNP (12 seedlings) was added. After an additional 3 hr in the dark, the bathing solns were collected, their vols determined, and samples taken for sugar analysis. From the third group of 12 seedlings, the scutella were excised (at the end of the fructose incubation) into 10 ml of H_2O contained in a 25 ml conical flask. The flask was incubated at 30° and samples taken for sugar analysis at 30 min intervals for 3 hr. 12 scutella (minus root–shoot axes) weighed between 1 and 1.2 g.

The results shown in Table 2 indicate that sucrose leakage into DNP represents an efflux of sucrose through the plasmalemma. In this experiment, entire seedlings (minus endosperm) were placed with the scutella, but not the root-shoot axes, immersed in M fructose. After 3 hr, the fructose was replaced with water or 0.5 mM DNP. With another group of seedlings, the scutella were excised and placed in water at the end of the fructose incubation. The amounts of sucrose that leaked from these three groups of scutella are shown in Table 2. Only a small amount of sucrose leaked into water when the root–shoot axis was attached, although a rapid leakage (completed in 90 min) occurred when it was removed. Evidence has been presented [16] that sucrose leakage that occurs upon removal of the seedling axis (when the main vascular bundle leading from the scutellum to the axis is cut) leaves the scutellum through the cut ends of the phloem. In contrast, considerable sucrose leaked from the scutella into DNP when the root–shoot axes were attached (i.e. when the only exit to the bathing solution was through the plasmalemma).

Partial separation of the process releasing stored sucrose from the process causing sucrose leakage could be achieved by incubating slices in DNP plus M sorbitol (Fig. 4). In this experiment, slices were incubated in M fructose for 2 hr and then in M sorbitol for 30 min. Then the slices were placed in water, sorbitol or sorbitol plus DNP (time zero on graph). Upon direct transfer to water, about 40 μmol sucrose leaked from the slices, and a similar amount leaked from slices transferred to water after an additional 30 min in sorbitol (Fig. 4a). This leakage was of the type shown in Table 1, and the amount

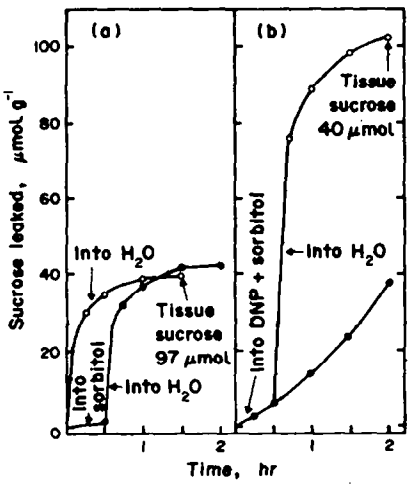

Fig. 4. Effect of sorbitol on DNP-induced sucrose efflux. Slices were incubated for 2 hr in M fructose followed by 30 min in M sorbitol. Then they were transferred (time zero on graph) to 17 ml H_2O, M sorbitol or M sorbitol plus 0.5 mM DNP. Slices in sorbitol or sorbitol plus DNP were transferred to H_2O at the times indicated on the graph. During incubation in sorbitol plus DNP, the pH was maintained at 4 by the addition of 20 mM HCl in M sorbitol at 10 min intervals. At time zero, the slices contained 139 μmol sucrose/g.

Table 3. A comparison of water-treated and acid-treated slices*

Measurement	Treatment H_2O	HCl
Cations removed during treatment (μmol/g)		
Mg^{2+}	3	13.5
Ca^{2+}	0.5	2.3
K^+	2.3	13.3
Metabolic activity after treatment (μmol/g hr)		
O_2 uptake	46	44.2
sucrose uptake	30.6	35.7
sucrose synthesis	29.5	31.5

* Slices were incubated at 30° in 10 ml H_2O or HCl (10 mM) for 30 min, and then the bathing solns were collected for cation analysis. Mg^{2+} and Ca^{2+} were determined by atomic absorption spectrophotometry and K^+ by flame emission spectrophotometry. After the intial incubations in H_2O or HCl, the slices were washed and incubated in H_2O for 30 min before measuring metabolic activity. O_2 uptake measurements were done at 30° using 0.2 g of slices and 2 ml of H_2O in Warburg vessels. For sucrose uptake, groups of slices (1 g) were incubated at 30° in 10 ml of 50 mM sucrose for 4 hr. For sucrose synthesis, slices (1 g) were placed in 10 ml of 0.1 M fructose for 2 hr.

of leakage can be taken as an estimate of the size of the leakable sucrose pool at the end of the fructose incubation [15, 22]. When slices were transferred to sorbitol plus DNP and then to water, 102 μmol sucrose leaked into the bathing solution (Fig. 4b). The extra sucrose (62 μmol) apparently was released from storage into the leakable pool during the 30 min DNP plus sorbitol incubation. During the 2 hr incubation in sorbitol plus DNP (Fig. 4b), sorbitol inhibited sucrose efflux across the plasemalemma (cf. Fig. 3, sucrose efflux from the high-sucrose slices), but it apparently did not inhibit sucrose release from storage into the leakable pool.

The results of this section (Table 2, Figs. 3 and 4) indicate that DNP causes an efflux of sucrose across the plasmalemma, and that the sucrose originates from two compartments of the cell connected in series.

Effect of pH on sucrose efflux

DNP rapidly accumulates in scutellum slices, reaching a peak after 15 to 20 min of incubation; and then there is an efflux of DNP [21]. The amount of DNP in the tissue is, in part, a function of the bathing solution pH [21]. Therefore, in order to study the effect of pH on sucrose efflux it was necessary to incubate groups of slices in DNP for a short period at a single pH and then to transfer the slices to water and maintain different pH's during sucrose efflux. It was also necessary to shorten the lag period between addition of DNP and development of steady rates of sucrose efflux (cf. Fig. 3). This was accomplished by using slices that had been incubated in 10 mM HCl for 30 min. The acid incubation was not a drastic treatment, and this is illustrated in Table 3 where acid-treated and water-treated slices are compared. Acid treatment removed considerable amounts of K^+ and Mg^+ and a small amount of Ca^{2+}, but it did not appreciably change the rate of O_2 uptake nor did it impair the ability of slices to take up sucrose or to synthesize sucrose from exogenous fructose. Fig. 5 shows the time course of sucrose efflux from acid-treated slices placed in DNP maintained at pH 4. There was only a 10 min lag period before sucrose efflux reached a high steady rate. The lag period could be increased to about 30 min by incubation of acid-treated slices in a solution of $MgCl_2$, $CaCl_2$ and KCl (Fig. 5), but the full 40 to 50 min lag period of water-treated slices (Fig. 3) was not restored. Fig. 5 also shows that less than 2 μmol of sucrose leaked from acid-treated slices placed in water, another indication that acid treatment is not a drastic treatment.

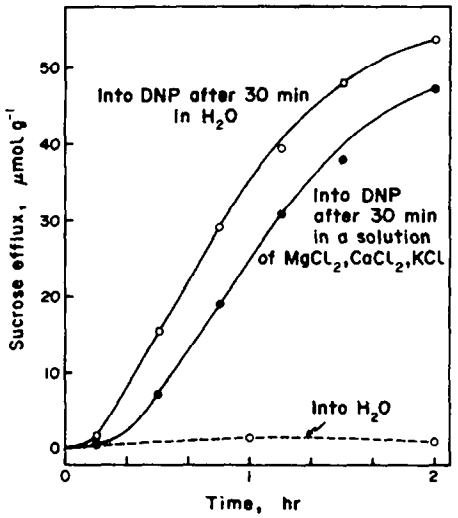

Fig. 5. Sucrose efflux from acid-treated slices. Slices (1 g) were incubated in 10 mM HCl for 30 min, and then incubated for an additional 30 min in either H_2O or a soln of $MgCl_2$, $CaCl_2$ and KCl (each at a concn of 20 mM). The slices were washed and placed (time zero) in 17 ml of 0.5 mM DNP maintained at pH 4 by adding HCl at 10 min intervals. One groups of slices (acid-treated but not salt-treated) was placed in H_2O maintained at pH 4 to serve as a control. At zero time, the slices contained 70 μmol sucrose per g.

To measure the effect of pH on sucrose efflux, three groups of acid-treated slices were placed in DNP (pH 3.9) for 10 min after which the DNP solution was replaced with water and the pH was maintained at 3.5, 4 or 5 with HCl. The time courses of sucrose efflux, DNP fluxes and H^+ influx at these pH's are shown in Fig. 6.

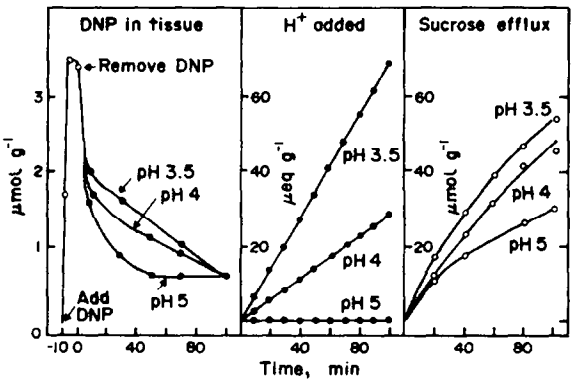

Fig. 6. Effect of pH on DNP and sucrose efflux and on H^+ influx. Acid-treated slices were incubated in 17 ml of 0.5 mM DNP (initial pH, 3.9). After 10 min the DNP was removed and 17 ml of H_2O was added (time zero on graph). The pH was maintained by the addition of 20 mM HCl at 10 min intervals. Samples of the bathing soln were taken for DNP and sucrose analyses at the time shown.

H^+ influx was greatly influenced by pH; at pH 5 there was no net influx, whereas at pH 3.5 influx occured at a rate of 40 μeq/hr. Sucrose efflux also increased as the pH was lowered, but the effect was not as pronounced as that for H^+ influx. DNP content of the slices declined during the course of the experiment, the rate of decline being greater as the pH was raised. Although higher rates of H^+ and sucrose fluxes were associated with higher DNP contents, there is no clear correlation between them. Clearly, pH had a direct effect on H^+ and sucrose fluxes over and above its effect on DNP content.

Effect of mannose treatment on H^+ and sucrose fluxes

Incubation in mannose causes the ATP level of scutellum slices to drop to less than 12% (<0.05 μmol/g fr. wt) of that in water incubated slices without a concomitant rise in ADP [23]. Mannose treatment also inhibits H^+ efflux [21]. For these reasons it was of interest to measure sucrose and H^+ influxes in mannose-treated slices. Acid-treated slices were incubated in 50 mM mannose for 30 min at 30°, and then they were washed and placed in water or 0.5 mM DNP. Both bathing solutions were maintained at pH 4 with HCl. During 2 hr in water it was necessary to add only 3.7 μeq HCl, and only 2 μmol sucrose leaked from the slices. In DNP, on the other hand, 50 μmol sucrose leaked from the mannose-treated slices (cf. Fig. 5), and this was accompanied by the influx of 49 μeq H^+.

DISCUSSION

The results of this paper indicate that sucrose can be accumulated in two compartments of the scutellum cell: the leakable pool and the stored pool. (Fig. 1 and 2, Table 1). In fresh slices, about 90% of the sucrose was contained in the stored pool (Fig. 2, zero time). During incubation of these slices in 0.1 M fructose almost all of the newly synthesized sucrose was accumulated in the stored pool (Table 1), and as the amount of sucrose in the tissue increased the rate of sucrose accumulation decreased (Fig. 1). A high rate of sucrose accumulation in the leakable pool was obtained by incubating slices in M fructose or glucose, a condition that would rarely occur in nature but one that, nevertheless, was useful in clearly identifying the presence of two sucrose compartments. A constant rate of sucrose accumulation in the leakable pool was obtained over a 7 hr period (Fig. 2). Apparently, the sucrose concentration of the leakable pool did not reach levels inhibitory to sucrose synthesis, a consequence, perhaps, of the low amount of sucrose initially present in this pool.

The location of the leakable pool has been assigned to the cytoplasm on the basis of two experimental observations: (1) hexose phosphates are released along with sucrose from scutella transferred from hexose or hexitol to water [24]. (2) Sucrose of the leakable pool is metabolized before sucrose of stored pool [22]. Because of this latter observation and because a large amount of stored sucrose most likely requires a large storage compartment, it is presumed that together the vacuoles of these multivacuolate cells make up the storage compartment [22].

These assignments of location of the sucrose pools are tentative. If they are correct, DNP-induced sucrose efflux represents transport across both tonoplast and plasmalemma. Sucrose efflux might result if DNP caused (1) membrane injury, (2) a low ATP level, (2) a high ADP level, (4) collapse of a pH gradient and/or a membrane potential, (5) a lowering of cytoplasmic pH or (6) if DNP reacted directly with the membrane transport system. (1) It is unlikely that sucrose efflux indicates membrane injury since DNP can be washed from the slices which then regain their ability to synthesize sucrose and to hold that sucrose within the cells [21]. (2) Although mannose treatment drastically reduces the ATP content of slices [23], it did not induce H^+ influx or sucrose efflux. It does, however, inhibit the reverse processes; sucrose uptake is strongly inhibited [23] and H^+ efflux is completely inhibited at pH 5.7 [21]. Evidently, sucrose efflux and H^+ influx did not result from a low level of ATP. (3) If energy coupling during sucrose accumulation involves a readily reversible reaction of ATP with the membrane transport system, a high ADP and a low ATP level might result in efflux of sucrose coupled to ATP synthesis. DNP, by uncoupling mitochrondrial oxidative phosphorylation, could maintain a high ADP/ATP ratio. The results with mannose do not preclude this idea since mannose reduces the ATP level without a concomitant rise in ADP [23]. (4) West and Mitchell [2] present evidence that galactoside transport in *E. coli* is a proton symport, and they suggest that accumulation of lactose requires a pH difference across the membrane that is maintained by a proton pump. In addition, it has been suggested that a potential difference acoss the membrane, also maintained by a proton pump, might act in concert with a pH difference to drive sugar transport [25, 26]. Uncouplers of oxidative phosphorylation supposedly cause dissipation of the pH gradient and membrane potential, thereby releasing accumulated sugar.

As yet, there is no evidence that sucrose uptake is linked to H^+ transport in the scutellum. The results of this paper do not indicate that sucrose efflux resulted from an uncoupler-induced dissipation of a pH gradient since the rate of efflux increased as the gradient was increased, i.e. as the pH was lowered (Fig. 6). At pH 5 there was sucrose efflux but no net H^+ influx (Fig. 6). Therefore, it also appears unlikely that a sucrose–H^+ antiport was operative during sucrose efflux. The situation at the tonoplast is not known. If sucrose accumulation in the vacuole is driven by the pH gradient across the tonoplast, a sucrose–H^+ antiport would appear to be necessary.

If there is a potential across the plasmalemma of scutellum cells, it probably represents (according to the calculations of Nobel [27]) less than 1 neq of excess negative charge per g fr. wt of tissue, assuming inside the cell is negative. It was suggested before [21] that DNP-induced H^+ influx resulted from reversal of a H^+ pump in the plasmalemma. Such a reversal should also rapidly dissipate the membrane potential. However, it is not clear, in the absence of cotransport of an ion, how a membrane potential could support sucrose accumulation. (5) The efflux of DNP and influx of H^+ indicate that DNP causes an acidification of the cytoplasm (Fig. 6, ref. [21]). Furthermore, DNP influx followed by DNP efflux occurs at pH 5 or in the presence of Ca^{2+} when the H^+ influxes from the bathing solution are small [21]. This indicates that acidification of the cytoplasm can also occur by H^+ influx from the vacuoles. Why sucrose efflux should occur upon acidification of the cytoplasm

is not clear. One possibility is that sucrose and H^+ are cotransported (an antiport system at the tonoplast and a symport system at the plasmalemma) under the influence of an electrical potential, the cytoplasm being negative with respect to both bathing solution and vacuole. If such a system were operative, sucrose efflux would occur upon collapse of the potential and acidification of the cytoplasm. (6) If energy coupling during sucrose transport involves redox reactions taking place within the plasmalemma and tonoplast, DNP might react directly at sites within these membranes, dissipating, in some way, the energized state and thereby allowing the sucrose concentration to equalize across the membrane.

It is assumed in the above discussion that during efflux sucrose is transported over part, at least, of the system utilized for sucrose uptake, Although sucrose exhange across the plasmalemma of scutellum cells has been observed [18] there is evidence that the sucrose uptake mechanism of the scutellum plasmalemma does not release free sucrose or free hexose into the cell [17, 22].

EXPERIMENTAL

Maize grains (*Zea mays* L., cv. G4455 or G4949, Louisiana Seed Co., Inc. Alexandria, Louisiana, USA) were soaked in running tap H_2O for 24 hr and then placed on moist paper towels in the dark at 24–25° for 72 hr. The scutella were excised and cut transversely into slices 0.5 mm or less in thickness. The slices were washed in H_2O until the washings were clear, blotted on filter paper and weighed in groups of 0.5 g or 1 g. Acid-treated slices were prepared by incubating slices (1 g) in 10 mM HCl (10 ml) at 30° for 30 min. At the end of the HCl incubation, the slices were washed twice with H_2O (10 ml) and then incubated in H_2O for an additional 30 min. When entire seedlings were used, the endosperm was carefully removed to avoid cutting the scutellum or injuring the roots. Seedlings were washed, blotted on filter paper and used immediately. Except for the expt of Table 2, all incubations were carried our at 30°. DNP content of the slices was determined as described previously [21]. Methods for the analysis of sugars in scutellum tissue and in bathing solns have been described [12]. Efflux of sucrose under the influence of DNP was accompanied by the appearance in the bathing soln of glucose and fructose in *ca* equal amounts. It is assumed that sucrose efflux preceded sucrose hydrolysis because exogenous sucrose is hydrolyzed in the presence of DNP [17]. In this paper, therefore, sugar efflux (sucrose + glucose) is reported as sucrose efflux.

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